

**WHAT IS CLAIMED IS:**

1. A method of eliciting an immune response in a human individual comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein 70 noncovalently bound to an antigenic molecule.
2. A method of eliciting an immune response in a human individual comprising administering to the individual a composition comprising an amount of a complex in the range of 50 to 5,000 micrograms, said complex consisting essentially of a heat shock protein 90 noncovalently bound to an antigenic molecule.
3. A method of eliciting an immune response in a human individual comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to an antigenic molecule.
4. The method according to claim 1, 2 or 3 in which the individual has liver cancer, colon cancer, or breast cancer.
5. The method according to claim 1 in which the amount of the complex is in the range of 10 to 100 micrograms.
6. The method according to claim 2 in which the amount of the complex is in the range of about 100 micrograms.
7. The method according to claim 3 in which the amount of the complex is in the range of 10 to 100 micrograms.

8. The method according to claim 1, 2 or 3, further comprising administering to the individual an effective amount of a biological response modifier selected from the group consisting of interferon- $\alpha$ , interferon- $\gamma$ , interleukin-5, interleukin-4, interleukin-6, and tumor necrosis factor.

9. The method according to claim 1, 2 or 3 in which said administering step is repeated at weekly intervals.

10. The method according to claim 1, 2 or 3 in which said complex is administered intramuscularly, subcutaneously, intraperitoneally or intravenously.

11. The method according to claim 1, 2 or 3 in which said administering step is repeated five times, the first administration being on the left arm, the second administration being on the right arm, the third administration being on the left belly, the fourth administration being on the right belly, the fifth administration being on the left thigh, and the sixth administration being on the right thigh; said first through sixth administration being subcutaneously.

12. A method of treating a human individual having cancer, comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein noncovalently bound to an antigenic molecule.

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13. A method of treating a human individual having cancer, comprising administering to the individual a composition comprising an amount of a complex in the range of 50 to 5,000 micrograms, said complex consisting essentially of a heat shock protein noncovalently bound to an antigenic molecule.

14. A method of treating a human individual having cancer, comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to an antigenic molecule.

15. The method according to claim 12, 13 or 14 in which the cancer comprises a sarcoma or carcinoma, selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

16. The method according to claim 12, in which the amount of the complex is in the range of 10 to 100 micrograms.

17. The method according to claim 13 in which the amount of the complex is in the range of about 100 micrograms.

18. The method according to claim 14 in which the amount of the complex is in the range of 10 to 100 micrograms.

5 19. The method according to claim 12, 13 or 14 in which the complex is prepared from cancerous tissue autologous to the individual.

20. The method according to claim 12, 13 or 14 in which  
10 the complex is prepared from cancerous tissue allogeneic to the individual.

21. The method according to claim 12, 13 or 14, further comprising administering to the individual an effective  
15 amount of a biological response modifier selected from the group consisting of interferon- $\alpha$ , interferon- $\gamma$ , interleukin-2, interleukin-4, interleukin-6, and tumor necrosis factor.

22. The method according to claim 12, 13 or 14 in which  
20 said administering step is repeated at weekly intervals.

23. The method according to claim 16, 17 or 18 in which said administering step is repeated five times, the first administration being on the left arm, the second  
25 administration being on the right arm, the third administration being on the left belly, the fourth administration being on the right belly, the fifth administration being on the left thigh, and the sixth administration being on the right thigh; said first through  
30 sixth administration being subcutaneously.

24. A method of treating a human individual having cancer comprising:

35 (a) administering to the individual a composition comprising about 25 micrograms of a complex, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to a

peptide, said complex having been isolated from cancerous tissue of said individual; and  
(b) repeating said administering of step (a) at weekly intervals for five weeks, the first administration being on the left arm, the second administration being on the right arm, the third administration being on the left belly, the fourth administration being on the right belly, the fifth administration being on the left thigh, and the sixth administration being on the right thigh; said first through sixth administration being subcutaneously.

25. A method of preventing cancer in a human individual in whom prevention of cancer is desired comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein 70 noncovalently bound to an antigenic molecule.

26. A method of preventing cancer in a human individual in whom prevention of cancer is desired, comprising administering to the individual a composition comprising an amount of a complex in the range of 50 to 5,000 micrograms, said complex consisting essentially of a heat shock protein 90 noncovalently bound to an antigenic molecule.

27. A method of preventing cancer in a human individual in whom prevention of cancer is desired, comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to an antigenic molecule.

28. The method according to claim 25, in which the amount of the complex is in the range of 10 to 100 micrograms.

29. The method according to claim 26, in which the amount of the complex is in the range of 100 micrograms.

30. The method according to claim 27, in which the amount of the complex is in the range of 10 to 100 micrograms.

31. The method according to claim 12, 13, or 14 in which the antigenic molecule is a peptide with which the heat shock protein is endogenously associated *in vivo*, and the complex is isolated from cancerous tissue.

32. The method according to claim 31 in which the cancerous tissue is from the individual.

33. The method according to claim 12, 13, or 14 in which the noncovalent complex of the heat shock protein and antigenic molecule is produced *in vitro*.

34. The method according to claim 33 in which the antigenic molecule is a tumor-specific antigen.

35. The method according to claim 25, 26, or 27 in which the antigenic molecule is a peptide with which the heat shock protein is endogenously associated *in vivo*.

36. A method of treating or preventing an infectious disease in a human individual in whom such treatment or prevention is desired comprising administering to the individual a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein noncovalently bound to an antigenic molecule.

37. A method of treating or preventing an infectious disease in a human individual in whom such treatment or prevention is desired comprising administering to the

individual a composition comprising an amount of a complex in the range of 50 to 5,000 micrograms, said complex consisting essentially of a heat shock protein 90 noncovalently bound to an antigenic molecule.

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38. A method of treating or preventing an infectious disease in a human individual in whom such treatment or prevention is desired comprising administering to the individual a composition comprising an amount of a complex in 10 the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to an antigenic molecule.

39. The method according to claim 36 in which the 15 amount of the complex is in the range of 10 to 100 micrograms.

40. The method according to claim 37 in which the amount of the complex is in the range of about 100 20 micrograms.

41. The method according to claim 38 in which the amount of the complex is in the range of 10 to 100 micrograms.

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42. The method according to claim 36, 37, or 38 in which the antigenic molecule is a peptide with which the heat shock protein is endogenously associated in cells infected with an infectious agent that causes the infectious disease.

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43. The method according to claim 36, 37, or 38 in which the antigenic molecule is an antigen of an infectious agent that causes the infectious disease.

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44. The method according to claim 43 in which the infectious agent is a virus, bacterium, protozoa, fungus, or parasite.

45. A method for measuring tumor rejection in vivo in an individual having a tumor comprising measuring the generation by the individual of MHC Class I-restricted CD8<sup>+</sup> cytotoxic T lymphocytes specific to the tumor.

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46. A kit comprising in a container a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein 70 noncovalently bound to an antigenic molecule.

47. A kit comprising in a container a composition comprising an amount of a complex in the range of 50 to 5,000 micrograms, said complex consisting essentially of a heat shock protein 90 noncovalently bound to an antigenic molecule.

48. A kit comprising in a container a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein gp96 noncovalently bound to an antigenic molecule.

49. A kit comprising a plurality of containers, each container having a composition comprising an amount of a complex in the range of 10 to 600 micrograms, said complex consisting essentially of a heat shock protein 70 noncovalently bound to an antigenic molecule.

50. The kit of claim 46 in which the amount of the complex is in the range of 10 to 100 micrograms.

51. The kit of claim 47 in which the amount of the complex is in the range of about 100 micrograms.

52. The kit of claim 48 in which the amount of the complex is in the range of 10 to 100 micrograms.



53. A method of purifying hsp70-peptide complexes comprising:

- 5 (a) contacting a sample containing cellular proteins with ADP affixed to a solid substrate under conditions such that hsp70 in the sample can bind to the ADP; and
- (b) eluting the hsp70 bound to the ADP in step (a).

10 54. The method according to claim 53 wherein the contacting is carried out by column chromatography over ADP-agarose.

55. The method according to claim 53 wherein the cell 15 is a tumor cell.

56. The method according to claim 53 wherein the cell is infected with a virus.

20 57. The method according to claim 53 wherein the cell is infected with a bacterium.

58. The method according to claim 53 wherein the cell is infected with a protozoa.

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59. The method according to claim 53 wherein the cell is infected with a parasite.

Sub B1 60. A method of purifying hsp70-peptide complexes from 30 a cell comprising:

- (a) homogenizing the cell with a hypotonic buffer solution to produce a cell lysate;
- (b) centrifuging the cell lysate to obtain a supernatant;
- 35 (c) running the supernatant over an ADP-agarose column;

- (d) washing the ADP-agarose column with a buffer containing ADP; and  
(e) collecting the hsp70-peptide complex.

5 61. A method of purifying hsp70-peptide complexes comprising:

- 10 (a) contacting a sample containing cellular proteins with a nonhydrolyzable analog of ATP affixed to a solid substrate under conditions such that hsp70 in the sample can bind to the nonhydrolyzable analog of ATP; and  
15 (b) eluting the hsp70 bound to the nonhydrolyzable analog of ATP in step (a).

add  
A4

add  
B5

add  
C6

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